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(54) METHODS OF CONTROLLING CUTWORM PESTS

VERFAHREN ZUR BEKÄMPFUNG VON NACHTFALTERLARVEN

PROCEDES DESTINES A LA LUTTE CONTRE LE VER-GRIS

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(56) References cited:
WO-A-91/16434 **WO-A-94/05771**
WO-A-98/18932 **WO-A-99/24581**

- **J.MÜLLER-COHN ET AL.: "Spodoptera littoralis (Lepidoptera: Noctuidae) Resistance to CryIC and Cross-Resistance to Other Bacillus thuringiensis Crystal Toxins" JOURNAL OF ECONOMIC ENTOMOLOGY., vol. 89, no. 4, August 1996 (1996-08), pages 791-796, XP002154101 ENTOMOLOGICAL SOCIETY OF AMERICA. COLLEGE PARK, MARYLAND., US ISSN: 0022-0493**
- **DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; H.S.SALAMA ET AL.: "The use of Bacilus thuringiensis to control two lepidopterous insect pests (Agrotis ypsilon and and Spodoptera littoralis)" retrieved from EPOQUE, accession no. prev199598279392 XP002154102 & AZEIGER FÜR SCHÄDLINGSKUNDE PFLANZENSCHUTZ UMWELTSCHUTZ, vol. 68, 1995, pages 15-17,**

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Description

Background of the Invention

[0001] Insects and other pests cost farmers billions of dollars annually in crop losses and in the expense of keeping these pests under control. The losses caused by insect pests in agricultural production environments include decrease in crop yield and reduced crop quality. Increased harvesting costs are also a result of insect infestations.

[0002] Insect problems have been partially addressed by cultivation methods, such as crop rotation, and by fertilizing with high levels of phosphate to stimulate the growth of strong root systems by the plants. However, crop rotation can be disrupted by, for example, an emerging two-year diapause (or overwintering) trait of northern corn rootworms. Chemical insecticides are relied upon most heavily to guarantee the desired level of control.

[0003] Chemical pesticides have provided an effective method of pest control. However, the public has become concerned about the amount of residual chemicals which might be found in food, ground water, and elsewhere in the environment. Therefore, synthetic chemical pesticides are being increasingly scrutinized for their potential adverse environmental consequences. Some synthetic chemical pesticides can poison the soil and underlying aquifers, pollute surface waters as a result of runoff, and destroy non-target life forms. Some synthetic chemical control agents have the further disadvantage of presenting public safety hazards when they are applied in areas where pets, farm animals, or children may come into contact with them. They can also pose health hazards to the people applying them, especially if the proper application techniques are not followed. Regulatory agencies around the world are restricting and/or banning the uses of many synthetic pesticides, particularly those that are persistent in the environment and that enter the food chain. Stringent new restrictions on the use of pesticides and the elimination of some effective pesticides from the market place could limit economical and effective options for controlling costly pests.

[0004] Because of the problems associated with the use of many synthetic chemical pesticides, there exists a clear need to limit the use of these agents and to identify alternative control agents. The replacement of synthetic chemical pesticides, or the combination of these agents with biological pesticides, could reduce the levels of toxic chemicals in the environment.

[0005] A biological pesticidal agent that is being used with increasing popularity is the soil microbe *Bacillus thuringiensis* (*B.t.*). The soil microbe *Bacillus thuringiensis* (*B.t.*) is a Gram-positive, spore-forming bacterium. Most strains of *B.t.* do not exhibit pesticidal activity. Some *B.t.* strains produce pesticidal parasporal protein inclusions. These protein inclusions often appear microscopically as distinctively shaped crystals. The shape and type of the crystal inclusions can be used to characterize *B.t.* strains. The "δ-endotoxins" present in the crystalline inclusions, which typically have specific pesticidal activity, are different from exotoxins, which have a non-specific host range.

[0006] Commercial use of *B.t.* pesticides was originally restricted to a narrow range of lepidopteran (caterpillar) pests. For example, preparations of the spores and crystals of *B. thuringiensis* subsp. *kurstaki* have been used for many years as commercial insecticides for lepidopteran pests. More recently, however, investigators have discovered *B.t.* pesticides with specificities for a much broader range of pests. For example, *B.t. israelensis* and *morrisoni* have been used commercially to control insects of the orders Diptera and Coleoptera, respectively (Gaertner, F.H. [1989] "Cellular Delivery Systems for Insecticidal Proteins: Living and Non-Living Microorganisms," in *Controlled Delivery of Crop Protection Agents*, R.M. Wilkins, ed., Taylor and Francis, New York and London, 1990, pp. 245-255).

[0007] New subspecies of *B.t.* have now been identified, and genes responsible for active δ-endotoxin proteins have been isolated and sequenced (Höfte, H., H.R. Whiteley [1989] *Microbiological Reviews* 52(2):242-255). Höfte and Whiteley classified *B.t.* crystal protein genes into four major classes. The classes were *cry*I (Lepidoptera-specific), *cry*II (Lepidoptera- and Diptera-specific), *cry*III (Coleoptera-specific), and *cry*IV (Diptera-specific). The discovery of strains specifically toxic to other pests has been reported (Feitelson, J.S., J. Payne, L. Kim [1992] *Bio/Technology* 10:271-275). For example, the designations CryV and CryVI have been proposed for two new groups of nematode-active toxins.

[0008] Many *Bacillus thuringiensis* δ-endotoxin proteins are composed of two functional segments. These "full-length" proteins are comprised of a protease-resistant core toxin that corresponds to about the first half (the N-terminal portion) of the protein molecule. The three-dimensional structure of a core segment of a CryIIIA *B.t.* δ-endotoxin is known, and it was proposed that all related toxins have that same overall structure (Li, J., J. Carroll, D.J. Ellar [1991] *Nature* 353:815-821). The second half (the C-terminal portion) of the molecule is often referred to as the "protoxin segment." The protoxin segment is believed to participate in toxin crystal formation (Arvidson, H., P.E. Dunn, S. Strand, A.I. Aronson [1989] *Molecular Microbiology* 3:1533-1534; Choma, C.T., W.K. Surewicz, P.R. Carey, M. Pozsgay, T. Raynor, H. Kaplan [1990] *Eur. J. Biochem.* 189:523-527). The full 130 kDa toxin molecule is typically processed to the resistant core segment by proteases in the insect gut. The protoxin segment may thus convey a partial insect specificity for the toxin by limiting the accessibility of the core to the insect by reducing the protease processing of the toxin molecule (Haider, M.Z., B.H. Knowles, D.J. Ellar [1986] *Eur. J. Biochem.* 156:531-540) or by reducing toxin solubility (Aronson, A.I., E.S. Han, W. McGaughey, D. Johnson [1991] *Appl. Environ. Microbial.* 57:981-986).

[0009] The 1989 nomenclature and classification scheme of Höfte and Whiteley was based on both the deduced

amino acid sequence and the host range of the toxin. That system was adapted to cover 14 different types of toxin genes which were divided into five major classes. A revised nomenclature scheme has been proposed which is based solely on amino acid identity (Crickmore *et al.* [1996] Society for Invertebrate Pathology, 29th Annual Meeting, IIIrd International Colloquium on *Bacillus thuringiensis,* University of Cordoba, Cordoba, Spain, September 1-6, 1996, abstract). The mnemonic "cry" has been retained for all of the toxin genes except cytA and cytB, which remain a separate class. Roman numerals have been exchanged for Arabic numerals in the primary rank, and the parentheses in the tertiary rank have been removed. Current boundaries represent approximately 95% (tertiary rank), 75% (secondary rank), and 48% (primary rank) sequence identity. Many of the original names have been retained, although a number have been reclassified. *See also* "Revisions of the Nomenclature for the *Bacillus thuringiensis* Pesticidal Crystal Proteins," N. Crickmore, D.R. Zeigler, J. Feitelson, E. Schnepf, J. Van Rie, D. Lereclus, J. Baum, and D.H. Dean, *Microbiology and Molecular Biology Reviews* (1998) Vol. 62:807-813; and Crickmore, Zeigler, Feitelson, Schnepf, Van Rie, Lereclus, Baum, and Dean, "*Bacillus thuringiensis* toxin nomenclature" (1999) http://www.biols.susx.ac.uk/Home/Neil_Crickmore/Bt/index.html. That system uses the freely available software applications CLUSTAL W and PHYLIP. The NEIGHBOR application within the PHYLIP package uses an arithmetic averages (UPGMA) algorithm.

[0010] Isolating novel *B.t.* isolates and their toxin genes, as well as determining the precise pesticidal range of the toxins, has been a slow empirical process. As a result of extensive research and resource investment, patents have issued for new *B.t.* isolates, toxins, and genes, and for new uses of known *B.t.* toxins and isolates. See Feitelson *et al., supra,* for a review. However, the discovery of new *B.t.* isolates and new uses of known *B.t.* isolates and toxins remains an empirical, unpredictable art.

[0011] Smulevitch *et al.* ([1991] *FEBS Lett.* 293:25-26), Gleave *et al.* ([1991] *JGM* 138:55-62), and Shevelev *et al.* ([1993] *FEBS Lett.* 336:79-82) describe the characterization of *Cry9* toxins active against lepidopterans. Lambert *et al.* (Lambert, B., L. Buysse, C. Decock, S. Jansens, C. Piens, B. Saey, J. Seurinck, K. van Audenhove, J. Van Rie, A. Van Vliet, M. Peferoen [1996] *Appl. Environ. Microbiol* 62(1):80-86), and Published PCT applications WO 94/05771 and WO 94/24264 also describe *B.t.* isolates and *Cry9*C toxins active against lepidopteran pests.

[0012] U.S. Patent Nos. 5.126,133; 5,188,960; 5,246,852; and 5,691,308 disclose a *Cry*1Fa toxin and gene (81IA) from *B.t.* isolate PS81I. U.S. Patent Nos. 5,527,883; 5,508,264; 5,827,514; and 5,840,554 relate to *Cry*1F chimeric toxins. WO 99/24581 discloses various plant-optimized *cry*1F genes. U.S. Patent No. 5,686,069 discloses a *Cry*1Fb toxin and gene from *B.t.* isolate PS91C2.

[0013] With the use of genetic engineering techniques, various approaches for delivering *B.t.* toxins to agricultural environments are under development. The cloning and expression of a *B.t.* crystal protein gene in *Escherichia coli* was described in the published literature more than 15 years ago (Schnepf, H.E., H.R. Whiteley [1981] *Proc. Natl. Acad. Sci. USA* 78:2893-2897.). U.S. Patent No. 4,448,885 and U.S. Patent No. 4,467,036 both disclose the expression of *B.t.* crystal protein in *E. coli.* Recombinant DNA-based *B.t.* products have been produced and approved for use, including the use of plants genetically engineered with *B.t.* genes for insect resistance and the use of stabilized, microbial cells as delivery vehicles *of B.t.* proteins. Various improvements have been achieved by modifying *B.t.* toxins and/or their genes. For example, U.S. Patent Nos. 5,380,831 and 5,567,862 relate to the production of synthetic insecticidal crystal protein genes having improved expression in plants. Thus, isolated *B.t.* endotoxin genes are becoming commercially valuable.

[0014] Obstacles to the successful agricultural use of *B.t.* toxins include the development of resistance to *B.t.* toxins by insects. In addition, certain insects can be refractory to the effects of *B.t.* The latter includes insects such as boll weevils and cutworms, which heretofore have demonstrated no apparent sensitivity to most *B.t.* δ-endotoxins.

[0015] Cutworms progress through several instars as larvae. Although seedling cutting by later instar larvae produces the most obvious damage and economic loss, leaf feeding commonly results in yield loss in crops such as maize. Upon reaching later instars (third to fourth, for example), larvae begin to cut plants and plant parts, especially seedlings. Because of the shift in feeding behavior, economically damaging populations may build up unexpectedly with few early warning signs. Large cutworms can destroy several seedlings per day, and a heavy infestation can remove entire stands of crops. Cutworms' nocturnal habit and behavior of burrowing into the ground also makes detection and treatment problematic.

[0016] The black cutworm (*Agrotis ipsilon* (Hufnagel); Lepidoptera: Noctuidae) is a serious pest of many crops including maize, cotton, cole crops (*Brassica*, broccoli, cabbages, Chinese cabbages), and turf. Secondary host plants include beetroots, *Capsicum* (peppers), chickpeas, faba beans, lettuces, lucerne, onions, potatoes, radishes, rape (canola), rice, soybeans, strawberries, sugarbeet, tobacco, tomatoes, and forest trees. In North America, pests of the genus *Agrotis* feed on clover, corn, tobacco, hemp, onion, strawberries, blackberries, raspberries, alfalfa, barley, beans, cabbage, oats, peas, potatoes, sweetpotatoes, tomato, garden flowers, grasses, lucerne, maize, asparagus, grapes, almost any kind of leaf, weeds, and many other crops and garden plants. Other cutworms in the Tribe Agrotini are pests, in particular those in the genus *Feltia* (*e.g., F. jaculifera* (Guenée); equivalent to *ducens subgothica*) and *Euxoa* (*e.g., E. messoria* (Harris), *E. scandens* (Riley), *E. auxiliaris* Smith, *E. detersa* (Walker), *E. tessellata* (Harris), *E. ochrogaster* (Guenée). Cutworms such as *Peridroma saucia* can also be significant pests. Citrus plants, for example, can

also be the target of cutworm attacks (the "citrus cutworm" *Xylomyges curialis* is an example).

[0017] Cutworms are also pests outside North America, and the more economically significant pests attack chickpeas, wheat, vegetables, sugarbeet, lucerne, maize, potatoes, turnips, rape, lettuces, strawberries, loganberries, flax, cotton, soybeans, tobacco, beetroots, Chinese cabbages, tomatoes, aubergines, sugarcane, pastures, cabbages, groundnuts, *Cucurbita,* turnips, sunflowers, *Brassica,* onions, leeks, celery, sesame, asparagus, rhubarb, chicory, greenhouse crops, and spinach. The black cutworm *A. ipsilon* occurs as a pest outside North America, including Central America, Europe, Asia, Australia, Africa, India, Taiwan, Mexico, Egypt, and New Zealand.

[0018] The main cutworm species in Argentina are *Agrotis malefida, Porosagrotis gypaetiana*, and *Agrotis ipsilon* (also *spelled ypsilon*). These cutworms attack corn, soybean, and sunflower cultivars, for example. These insects can seriously reduce the population of seedlings, and in cases of severe attacks, can totally destroy entire plots.

[0019] Cultural controls for *A. ipsilon* such as peripheral weed control can help prevent heavy infestations; however, such methods are not always feasible or effective. Infestations are very sporadic, and applying an insecticide prior to planting or at planting has not been effective in the past. Some baits are available for control of cutworms in crops. To protect turfgrass such as creeping bentgrass, chemical insecticides have been employed. Use of chemical pesticides is a particular concern in turf-covered areas (*e.g.*, golf greens, athletic fields, parks and other recreational areas, professional landscaping, and home lawns) because of the close contact the public has with these areas. Natural products (*e.g.*, nematodes and azadirachtin) generally perform poorly.

[0020] To date, *Bacillus thuringiensis* products for control black cutworm have not been widely used to due to their heretofore lack of sufficient effectiveness. The rapid feeding and burrowing behaviors of the cutworm have made them particularly difficult to control with current biologically based pest solutions. For example, *Cry*1A(b) toxins are basically ineffective against cutworms.

Brief Summary of the Invention

[0021] The subject invention relates to the surprising discovery that *Cry*1F proteins are active against cutworms such as the black cutworm (*Agrotis ipsilon*). Thus, the subject invention provides methods for controlling these pests wherein said method comprises contacting said pest with a pesticidal amount of a *Bacillus thuringiensis* toxin comprising at least a pesticidal portion of a *Cry*1F toxin. Therefore, the use of full-length, truncated, and chimeric *Cry*1F proteins and genes is included in the subject invention. In preferred embodiments, the *Cry*1F toxin is a *Cry*1Fa toxin. Wild-type and synthetic *Cry*1F proteins can be used according to the subject invention. Thus, the use of polynucleotides (and/or their complements, preferably their full complements) that hybridize with known *cry*1F genes, preferably the core-toxin encoding portions, are included in this invention. Plant-optimized polynucleotides are used in preferred embodiments.

[0022] The subject invention includes the use of transgenic hosts including plant hosts such as corn, cotton, and sunflower. In a preferred embodiment, the subject invention concerns plants cells transformed with at least one polynucleotide sequence of the subject invention such that the transformed plant cells express pesticidal proteins in tissues consumed by the target pests. Such transformation of plants can be accomplished using techniques well known to those skilled in the art and would typically involve modification of the gene to optimize expression of the toxin in plants.

Brief Description of the Drawings

[0023]

Figure 1 shows mass of intact wheat seedlings and relative damage ratings 24 hours after infestation with 4th instar black cutworm larvae and after various treatments.

Figure 2 shows the fresh weights of wheat seedlings infested with 3rd instar black cutworm larvae 48 hours after infestation and after various treatments.

Figures 3 and 4 illustrate the results discussed in Example 4.

Brief Description ofthe Sequences

[0024] **SEQ ID NO. 1** is a polynucleotide sequence for a full-length, plant-optimized *cry*1F/*cry*1A(b) hybrid gene designated 1F1AB-PO.

[0025] **SEQ ID NO. 2** is an amino acid sequence for a full-length, plant-optimized CryIF/CryIA(b) chimeric toxin. The 1F1AB-PO gene encodes this toxin.

[0026] **SEQ ID NO. 3** is a polynucleotide sequence for a truncated, plant-optimized *cry*1F gene designated 1F-T-PO.

[0027] **SEQ ID NO. 4** is an amino acid sequence for a truncated, plant-optimized CryIF toxin. The genes designated 1F-T-PO, 1F-7G-PO, and 1F-7Z-PO encode this toxin.

[0028] **SEQ ID NO. 5** is the native polynucleotide sequence of the wild-type, full length *B.t.* toxin gene designated

81IA (*cry*IFa).

[0029] **SEQ ID NO. 6** is the amino acid sequence of the full length, wild-type *B.t.* toxin designated 81IA (CryIFa).

[0030] **SEQ ID NO. 7** is a polynucleotide sequence for a gene designated 1F-7G-PO, which is optimized for expression in cotton.

[0031] **SEQ ID NO. 8** is a polynucleotide sequence for a gene designated 1F-7Z-PO, which is optimized for expression in maize.

Detailed Disclosure of the Invention

[0032] The subject invention relates to the surprising discovery that *Cry*1 F proteins are active against cutworms such as the black cutworm (*Agrotis ipsilon*). Particularly surprising are the findings that 3rd instar, and greater, larvae are controlled by the toxins and genes according to the subject invention. Controlling first and second instar cutworm larvae is much less important than controlling later instars. While it was known that *Cry*I toxins are active against lepidopterans generally, cutworms were thought to be recalcitrant to *B.t.* toxins generally, as discussed in more detail above in the Background section.

[0033] The subject invention includes the use of recombinant hosts and provides plant-optimized polynucleotide sequences These polynucleotide sequences include plant-optimized genes designated 1F1AB-PO, 1F-T-PO, 1F-7G-PO, and 1F-7Z-PO. These genes are disclosed in WO 99/24581. Preferred plant hosts include corn, soybeans, cotton, wheat, canola, and sunflower.

[0034] In some embodiments of the subject invention, genes encode a *Cry*IF toxin that is truncated compared to the full length *Cry*IF toxin. The truncated toxins of the subject invention are typically missing all or a portion of the protoxin segment. Also, the truncated genes of the subject invention can be used for the production of chimeric genes and proteins. One example is the plant-optimized gene comprising a *cry*IF portion and a *cry*IA(b) portion, wherein the hybrid gene encodes a chimeric toxin. Preferred chimeric genes and toxins are disclosed in U.S. Patent Nos. 5,527,883 and 5,840,554. Other chimeric genes and toxins, which can be used according to the subject invention, are disclosed in U.S. Patent Nos. 5,508,264 and 5,827,514. In a preferred embodiment, the *Cry*IF portion of the chimeric toxin is itself pesticidal. Fusion toxins can also be used according to the subject invention.

[0035] In a preferred embodiment, the subject invention concerns plants cells transformed with at least one polynucleotide sequence such that the transformed plant cells express pesticidal toxins in tissues consumed by the target pests which thereby contact the pesticidal protein. Such transformation of plants can be accomplished using techniques well known to those skilled in the art and would typically involve modification of the gene to optimize expression of the toxin in plants.

[0036] It should be apparent to a person skilled in this art that, given the sequences of the genes as set forth herein, the genes of the subject invention can be obtained through several means. In preferred embodiments, the subject genes may be constructed synthetically by using a gene synthesizer, for example. The specific genes exemplified herein can also be obtained by modifying, according to the teachings of the subject invention, certain wild-type genes (for example, by point-mutation techniques) from certain isolates deposited at a culture depository as discussed below. For example, a wild-type *cry*IF gene can be obtained from *B.t.* isolate PS81I. Likewise, the *cry*IA(b) portions of the hybrid genes of the subject invention can be produced synthetically or can be derived by modifying wild-type genes. CryIA(b) toxins and genes have been described in, for example, Höfte *et al.* (1986) *Eur. J. Biochem.* 161:273; Geiser *et al.* (1986) *Gene* 48:109; and Haider *et al.* (1988) *Nucleic Acids Res.* 16:10927. Clones and additional wild-type isolates are discussed in more detail, above, in the section entitled "Background of the Invention" and in the list, below.

[0037] Cultures discussed in this application have been deposited, in accordance with the Budapest Treaty, in the Agricultural Research Service Patent Culture Collection (NRRL), Northern Regional Research Center, 1815 North University Street, Peoria, Illinois 61604, USA. The deposited strains listed below are disclosed in the patent references as discussed above in the section entitled "Background of the Invention."

Subculture	Accession Number	Deposit Date
B.t. PS81I	NRRL B-18484	April 19, 1989
E. coli (NM522) (pMYC1603) (81IA)	NRRL B-18517	June 30, 1989

It should be understood that the availability of a deposit does not constitute a license to practice the subject invention in derogation of patent rights granted by governmental action.

[0038] Genes and toxins. The polynucleotides of the subject invention can be used to form complete "genes" to encode proteins or peptides in a desired host cell. For example, as the skilled artisan would readily recognize, some of the polynucleotides in the attached sequence listing are shown without stop codons. Also, the subject polynucleotides

can be appropriately placed under the control of a promoter in a host of interest, as is readily known in the art.

[0039] As the skilled artisan would readily recognize, DNA typically exists in a double-stranded form. In this arrangement, one strand is complementary to the other strand and vice versa. As DNA is replicated in a plant (for example) additional, complementary strands of DNA are produced. Thus, the subject invention includes the use of the exemplified polynucleotides shown in the attached sequence listing and the complementary strands thereof. The "coding strand" is often used in the art to refer to the strand that binds with the anti-sense strand. In order to express a protein *in vivo*, a strand of DNA is typically transcribed into a complementary strand of mRNA which is used as the template for translating the protein. The mRNA is actually transcribed from the "anti-sense" strand of DNA. The "sense" or "coding" strand has a series of codons (a codon is three nucleotides that can be read three-at-a-time to yield a particular amino acid) that can be read as an open reading frame (ORF) to form a protein or peptide of interest. RNA and PNA (peptide nucleic acids) that are functionally equivalent to the exemplified DNA are included in the subject invention.

[0040] Genes and toxins of the subject invention can be identified, obtained, and characterized by using oligonucleotide probes, for example. Probes are detectable nucleotide sequences. The specifically exemplified polynucleotides of the subject invention, including portions thereof that are sufficient to encode an active toxin, can, themselves, be used as probes. Probes may be DNA, RNA, or PNA (peptide nucleic acid). These sequences may be detectable by virtue of an appropriate label including or may be made inherently fluorescent, for example, as described in International Application No. WO93/16094. As is well known in the art, if the probe molecule and nucleic acid sample hybridize by forming a strong bond between the two molecules, it can be reasonably assumed that the probe and sample have substantial homology. Preferably, hybridization is conducted under stringent conditions by techniques well-known in the art, as described, for example, in Keller, G.H., M.M. Manak (1987) *DNA Probes,* Stockton Press, New York, NY., pp. 169-170. For example, as stated therein, hybridization conditions of high stringency can be achieved by first washing with 2x SSC (Standard Saline Citrate)/0.1% SDS (Sodium Dodecyl Sulfate) for 15 minutes at room temperature. Two washes are typically performed. Higher stringency can be achieved by lowering the salt concentration and/or by raising the temperature. For example, the above hybridization step can be followed by washing with 0.1x SSC/0.1% SDS for 15 minutes at room temperature, which in turn can be followed by washing with 0.1x SSC/0.1% SDS for 30 minutes at 55°C. The temperatures used for these steps can also be used with other protocols discussed herein (where SSPE is used in place of SSC, for example) as would be known in the art. The 2x SSC/0.1% SDS can be prepared by adding 50 ml of 20x SSC and 5 ml of 10% SDS to 445 ml of water. 20x SSC can be prepared by combining NaCl (175.3 g / 0.150 M), sodium citrate (88.2 g / 0.015 M), and water to 1 liter, followed by adjusting pH to 7.0 with 10 N NaOH. 10% SDS can be prepared by dissolving 10 g of SDS in 50 ml of autoclaved water, diluting to 100 ml, and aliquotting.

[0041] Detection of the probe provides a means for determining in a known manner whether hybridization has occurred. Such a probe analysis provides a rapid method for identifying toxin-encoding genes of the subject invention. The nucleotide segments which are used as probes according to the invention can be synthesized using a DNA synthesizer and standard procedures.

[0042] As used herein "stringent" conditions for hybridization refers to conditions which achieve the same, or about the same, degree of specificity of hybridization as the conditions employed by the current applicants. Specifically, hybridization of immobilized DNA on Southern blots with 32P-labeled gene-specific probes was performed by standard methods (Maniatis, T., E.F. Fritsch, J. Sambrook [1982] *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.). In general, hybridization and subsequent washes were carried out under stringent conditions that allowed for detection of target sequences. For double-stranded DNA gene probes, hybridization was carried out overnight at 20-25° C below the melting temperature (Tm) of the DNA hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. The melting temperature is described by the following formula (Beltz, G.A., K.A. Jacobs, T.H. Eickbush, P.T. Cherbas, and F.C. Kafatos [1983] *Methods of Enzymology*, R. Wu, L. Grossman and K. Moldave [eds.] Academic Press, New York 100:266-285).

[0043] Tm=81.5° C+16.6 Log[Na+]+0.41(%G+C)-0.61(%formamide)-600/length of duplex in base pairs.

[0044] Washes are typically carried out as follows:

(1) Twice at room temperature for 15 minutes in 1X SSPE, 0.1% SDS (low stringency wash).
(2) Once at Tm-20°C for 15 minutes in 0.2X SSPE, 0.1% SDS (moderate stringency wash).

[0045] For oligonucleotide probes, hybridization was carried out overnight at 10-20°C below the melting temperature (Tm) of the hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. Tm for oligonucleotide probes was determined by the following formula:

$$Tm\ (^{\circ}\ C) = 2(\text{number T/A base pairs}) + 4(\text{number G/C base pairs})$$

(Suggs, S.V., T. Miyake, E.H. Kawashime, M.J. Johnson, K. Itakura, and R.B. Wallace [1981] *ICN-UCLA Symp. Dev.*

Biol. Using Purified Genes, D.D. Brown [ed.], Academic Press, New York, 23:683-693).

[0046] Washes were typically carried out as follows:

(1) Twice at room temperature for 15 minutes 1X SSPE, 0.1% SDS (low stringency wash).

(2) Once at the hybridization temperature for 15 minutes in 1X SSPE, 0.1% SDS (moderate stringency wash).

[0047] Modification of genes and toxins. The genes and toxins useful according to the subject invention include not only the specifically exemplified sequences, but also include portions and/or fragments (including internal and/or terminal deletions compared to the full-length proteins), variants, mutants, substitutes (proteins having substituted amino acids), chimerics, and fusion proteins which retain the characteristic pesticidal activity of the proteins specifically exemplified herein. As used herein, the terms "variants" or "variations" of genes refer to nucleotide sequences which encode the same toxins or which encode equivalent toxins having pesticidal activity. As used herein, the term "equivalent toxins" refers to toxins having the same or essentially the same biological activity against the target pests as the claimed toxins.

[0048] Genes can be modified, and variations of genes may be readily constructed, using standard techniques. For example, techniques for making point mutations are well known in the art. Also, U.S. Patent No. 5,605,793, for example, describes methods for generating additional molecular diversity by using DNA reassembly after random fragmentation. Fragments of full-length genes can be made using commercially available endonucleases, and exonucleases can be used according to standard procedures. For example, enzymes such as *Bal*31 or site-directed mutagenesis can be used to systematically cut off nucleotides from the ends of these genes. Also, genes which encode active fragments may be obtained using a variety of restriction enzymes. Proteases may be used to directly obtain active fragments of these toxins.

[0049] Equivalent toxins and/or genes encoding these equivalent toxins can be derived from *B.t.* isolates and/or DNA libraries using the teachings provided herein. There are a number of methods for obtaining the pesticidal toxins of the instant invention. For example, antibodies to the pesticidal toxins disclosed and claimed herein can be used to identify and isolate other toxins from a mixture of proteins. Specifically, antibodies may be raised to the portions of the toxins which are most constant and most distinct from other *B.t.* toxins. These antibodies can then be used to specifically identify equivalent toxins with the characteristic activity by immunoprecipitation, enzyme linked immunosorbent assay (ELISA), or western blotting. Antibodies to the toxins disclosed herein, or to equivalent toxins, or fragments of these toxins, can readily be prepared using standard procedures in this art. The genes which encode these toxins can then be obtained from the microorganism.

[0050] Because of the redundancy of the genetic code, a variety of different DNA sequences can encode the same amino acid sequences. It is well within the skill of a person trained in the art to create these alternative DNA sequences encoding the same, or essentially the same, toxins. These variant DNA sequences are within the scope of the subject invention. As used herein, reference to "essentially the same" sequence refers to sequences which have amino acid substitutions, deletions, additions, or insertions which do not materially affect pesticidal activity. Fragments retaining pesticidal activity are also included in this definition.

[0051] Equivalent toxins will have amino acid similarity (and/or homology) with an exemplified toxin. The amino acid similarity/identity will typically be greater than 60%, preferably greater than 75%, more preferably greater than 80%, even more preferably greater than 90%, and can be greater than 95%. Preferred polynucleotides and proteins of the subject invention can also be defined in terms of more particular identity and/or similarity ranges. For example, the identity and/or similarity can be 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% as compared to a sequence exemplified herein. Unless otherwise specified, as used herein percent sequence identity and/or similarity of two nucleic acids is determined using the algorithm of Karlin and Altschul (1990), *Proc. Natl. Acad. Sci. USA* 87:2264-2268, modified as in Karlin and Altschul (1993), *Proc. Natl. Acad. Sci. USA* 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.* (1990), *J. Mol. Biol.* 215:402-410. BLAST nucleotide searches are performed witht he NBLAST program, score = 100, wordlength = 12, to obtain nucleotide sequences with the desired percent sequence identity. To obtain gapped alignments for comparison purposes, Gapped BLAST is used as described in Altschul *et al.* (1997), *Nucl. Acids Res.* 25:3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (NBLAST and XBLAST) are used. *See* http://www.ncbi.nih.gov. The identity scores can also be calculated using the methods and algorithms of Crickmore *et al.* as described in the Background section, above.

[0052] The amino acid homology will be highest in critical regions of the toxin which account for biological activity or are involved in the determination of three-dimensional configuration which ultimately is responsible for the biological activity. In this regard, certain amino acid substitutions are acceptable and can be expected if these substitutions are in regions which are not critical to activity or are conservative amino acid substitutions which do not affect the three-dimensional configuration of the molecule. For example, amino acids may be placed in the following classes: non-

polar, uncharged polar, basic, and acidic. Conservative substitutions whereby an amino acid of one class is replaced with another amino acid of the same type fall within the scope of the subject invention so long as the substitution does not materially alter the biological activity of the compound. Table 1 provides a listing of examples of amino acids belonging to each class.

Table 1.

Class of Amino Acid	Examples of Amino Acids
Nonpolar	Ala, Val, Leu, Ile, Pro, Met, Phe, Trp
Uncharged Polar	Gly, Ser, Thr, Cys, Tyr, Asn, Gln
Acidic	Asp, Glu
Basic	Lys, Arg, His

[0053] In some instances, non-conservative substitutions can also be made. The critical factor is that these substitutions must not significantly detract from the ability of plants to express the subject DNA sequences or from the biological activity of the toxin.

[0054] As used herein, reference to "isolated" polynucleotides and / or "purified" toxins refers to these molecules when they are not associated with the other molecules with which they would be found in nature; this would include their use in plants. Thus, reference to "isolated" and/or "purified" signifies the involvement of the "hand of man" as described herein.

[0055] While the subject invention provides specific embodiments of synthetic genes, other genes that are functionally equivalent to the genes exemplified herein can also be used to transform hosts, preferably plant hosts. Additional guidance for the production of synthetic genes can be found in, for example, U.S. Patent No. 5,380,831.

[0056] Recombinant hosts. The toxin-encoding genes of the subject invention can be introduced into a wide variety of microbial or plant hosts. In preferred embodiments, expression of the toxin gene results, directly or indirectly, in the intracellular production and maintenance of the pesticide. When transgenic/recombinant/transformed host cells are ingested by the pest, the pests will ingest the toxin. This is the preferred manner in which to cause contact of the pest with the toxin. The result is a control (killing or making sick) of the pest.

[0057] In some embodiments of the subject invention, transformed microbial hosts can be used in preliminary steps for preparing precursors, for example, that will eventually be used to transform, in preferred embodiments, plant cells and plants so that they express the toxins encoded by the genes of the subject invention. Microbes transformed and used in this manner are within the scope of the subject invention. Recombinant microbes may be, for example, *B.t.*, *E. coli,* or *Pseudomonas.*

[0058] The production of various recombinant organisms can be made by those skilled in the art using standard techniques. Materials necessary for these transformations are disclosed herein or are otherwise readily available to the skilled artisan. A wide variety of methods are available for introducing a *B.t.* gene encoding a toxin into the target host under conditions which allow for stable maintenance and expression of the gene. These methods are well known to those skilled in the art and are described, for example, in United States Patent No. 5,135,867, which is incorporated herein by reference.

[0059] The toxin-encoding genes of the subject invention can be introduced, via a suitable vector, into a wide variety of microbial and plant hosts. There are many crops of interest, such as corn, wheat, rice, cotton, soybeans, and sunflowers. Certain genes of the subject invention are particularly well suited for providing stable maintenance and expression, in the transformed plant, of the gene expressing the polypeptide pesticide, and, desirably, provide for improved protection of the pesticide from environmental degradation and inactivation. Expression of the toxin gene results, directly or indirectly, in the intracellular production and maintenance of the pesticide proteins. Thus, the target pest can contact the pesticidal proteins by ingesting plant tissue containing the pesticidal proteins, which are toxic to the pest. The result is control of the pest. Alternatively, suitable microbial hosts, *e.g., Pseudomonas fluorescens*, can be applied to the site of the pest, where some of which can proliferate, and are ingested by the target pests. The microbe hosting the toxin gene can be treated under conditions that prolong the activity of the toxin and stabilize the cell. The treated cell, which retains the toxic activity, then can be applied to the environment of the target pest.

[0060] Where the *B.t.* toxin gene is introduced via a suitable vector into a microbial host, and said host is applied to the environment in a living state, certain host microbes should be used. Microorganism hosts are selected which are known to occupy the "phytosphere" (phylloplane, phyllosphere, rhizosphere, and/or rhizoplane) of one or more crops of interest. These microorganisms are selected so as to be capable of successfully competing in the particular environment (crop and other insect habitats) with the wild-type microorganisms, provide for stable maintenance and expression of the gene expressing the polypeptide pesticide, and, desirably, provide for improved protection of the pesticide from environmental degradation and inactivation.

[0061] A large number of microorganisms are known to inhabit the phylloplane (the surface of the plant leaves) and/or the rhizosphere (the soil surrounding plant roots) of a wide variety of important crops. These microorganisms include bacteria, algae, and fungi. Of particular interest are microorganisms, such as bacteria, *e.g.*, genera *Pseudomonas, Erwinia, Serratia, Klebsiella, Xanthomonas, Streptomyces, Rhizobium, Rhodopseudomonas, Methylophilius, Agrobacterium, Acetobacter, Lactobacillus, Arthrobacter, Azotobacter, Leuconostoc*, and *Alcaligenes;* fungi, particularly yeast, *e.g.*, genera *Saccharomyces, Cryptococcus, Kluyveromyces, Sporobolomyces, Rhodotorula*, and *Aureobasidium.* Of particular interest are such phytosphere bacterial species as *Pseudomonas syringae, Pseudomonas fluorescens, Serratia marcescens, Acetobacter xylinum, Agrobacterium tumefaciens, Rhodopseudomonas spheroides, Xanthomonas campestris, Rhizobium melioti, Alcaligenes entrophus,* and *Azotobacter vinlandii*; and phytosphere yeast species such as *Rhodotorula rubra, R. glutinis, R. marina, R. aurantiaca, Cryptococcus albidus, C. diffluens, C. laurentii, Saccharomyces rosei, S. pretoriensis, S. cerevisiae, Sporobolomyces roseus, S. odorus, Kluyveromyces veronae,* and *Aureobasidium pollulans.* Of particular interest are the pigmented microorganisms.

[0062] A wide variety of ways are available for introducing a *B.t.* gene encoding a toxin into the target host under conditions which allow for stable maintenance and expression of the gene. These methods are well known to those skilled in the art and are described, for example, in United States Patent No. 5,135,867, which is incorporated herein by reference.

[0063] Treatment of cells. As mentioned above, *B.t.* or recombinant cells expressing a *B.t.* toxin can be treated to prolong the toxin activity and stabilize the cell. The pesticide microcapsule that is formed comprises the *B.t.* toxin within a cellular structure that has been stabilized and will protect the toxin when the microcapsule is applied to the environment of the target pest. Suitable host cells may include either prokaryotes or eukaryotes, normally being limited to those cells which do not produce substances toxic to higher organisms, such as mammals. However, organisms which produce substances toxic to higher organisms could be used, where the toxic substances are unstable or the level of application sufficiently low as to avoid any possibility of toxicity to a mammalian host. As hosts, of particular interest will be the prokaryotes and the lower eukaryotes, such as fungi.

[0064] The cell will usually be intact and be substantially in the proliferative form when treated, rather than in a spore form, although in some instances spores may be employed.

[0065] Treatment of the microbial cell, *e.g.*, a microbe containing the *B.t.* toxin gene, can be by chemical or physical means, or by a combination of chemical and/or physical means, so long as the technique does not deleteriously affect the properties of the toxin, nor diminish the cellular capability of protecting the toxin. Examples of chemical reagents are halogenating agents, particularly halogens of atomic no. 17-80. More particularly, iodine can be used under mild conditions and for sufficient time to achieve the desired results. Other suitable techniques include treatment with aldehydes, such as glutaraldehyde; anti-infectives, such as zephiran chloride and cetylpyridinium chloride; alcohols, such as isopropyl and ethanol; various histologic fixatives, such as Lugol iodine, Bouin's fixative, various acids and Helly's fixative (See: Humason, Gretchen L., *Animal Tissue Techniques,* W.H. Freeman and Company, 1967); or a combination of physical (heat) and chemical agents that preserve and prolong the activity of the toxin produced in the cell when the cell is administered to the host environment. Examples of physical means are short wavelength radiation such as gamma-radiation and X-radiation, freezing, UV irradiation, lyophilization, and the like. Methods for treatment of microbial cells are disclosed in United States Patent Nos. 4,695,455 and 4,695,462, which are incorporated herein by reference.

[0066] The cells generally will have enhanced structural stability which will enhance resistance to environmental conditions. Where the pesticide is in a proform, the method of cell treatment should be selected so as not to inhibit processing of the proform to the mature form of the pesticide by the target pest pathogen. For example, formaldehyde will crosslink proteins and could inhibit processing of the proform of a polypeptide pesticide. The method of treatment should retain at least a substantial portion of the bio-availability or bioactivity of the toxin.

[0067] Characteristics of particular interest in selecting a host cell for purposes of production include ease of introducing the *B.t.* gene into the host, availability of expression systems, efficiency of expression, stability of the pesticide in the host, and the presence of auxiliary genetic capabilities. Characteristics of interest for use as a pesticide microcapsule include protective qualities for the pesticide, such as thick cell walls, pigmentation, and intracellular packaging or formation of inclusion bodies; survival in aqueous environments; lack of mammalian toxicity; attractiveness to pests for ingestion; ease of killing and fixing without damage to the toxin; and the like. Other considerations include ease of formulation and handling, economics, storage stability, and the like.

[0068] Growth of cells. The cellular host containing the *B.t.* insecticidal gene may be grown in any convenient nutrient medium, preferably where the DNA construct provides a selective advantage, providing for a selective medium so that substantially all or all of the cells retain the *B.t.* gene. These cells may then be harvested in accordance with conventional ways. Alternatively, the cells can be treated prior to harvesting.

[0069] The *B.t.* cells of the invention can be cultured using standard art media and fermentation techniques. Upon completion of the fermentation cycle the bacteria can be harvested by first separating the *B.t.* spores and crystals from the fermentation broth by means well known in the art. The recovered *B.t.* spores and crystals can be formulated into

a wettable powder, liquid concentrate, granules or other formulations by the addition of surfactants, dispersants, inert carriers, and other components to facilitate handling and application for particular target pests. These formulations and application procedures are all well known in the art.

[0070] Formulations. Formulated bait granules containing an attractant and spores and crystals of the *B.t.* isolates, or recombinant microbes comprising the genes obtainable from the *B.t.* isolates disclosed herein, can be applied to the soil. Formulated product can also be applied as a seed-coating or root treatment or total plant treatment at later stages of the crop cycle. Plant and soil treatments *of B.t.* cells may be employed as wettable powders, granules or dusts, by mixing with various inert materials, such as inorganic minerals (phyllosilicates, carbonates, sulfates, phosphates, and the like) or botanical materials (powdered corncobs, rice hulls, walnut shells, and the like). The formulations may include spreader-sticker adjuvants, stabilizing agents, other pesticidal additives, or surfactants. Liquid formulations may be aqueous-based or non-aqueous and employed as foams, gels, suspensions, emulsifiable concentrates, or the like. The ingredients may include rheological agents, surfactants, emulsifiers, dispersants, or polymers.

[0071] As would be appreciated by a person skilled in the art, the pesticidal concentration will vary widely depending upon the nature of the particular formulation, particularly whether it is a concentrate or to be used directly. The pesticide will be present in at least 1% by weight and may be 100% by weight. The dry formulations will have from about 1-95% by weight of the pesticide while the liquid formulations will generally be from about 1-60% by weight of the solids in the liquid phase. The formulations will generally have from about 10^2 to about 10^4 cells/mg. These formulations will be administered at about 50 mg (liquid or dry) to 1 kg or more per hectare.

[0072] The formulations can be applied to the environment of the pest, *e.g.,* soil and foliage, by spraying, dusting, sprinkling, or the like.

[0073] Mutants. Mutants of the isolates of the invention can be made by procedures well known in the art. For example, an asporogenous mutant can be obtained through ethylmethane sulfonate (EMS) mutagenesis of an isolate. The mutants can be made using ultraviolet light and nitrosoguanidine by procedures well known in the art.

[0074] A smaller percentage of the asporogenous mutants will remain intact and not lyse for extended fermentation periods; these strains are designated lysis minus (-). Lysis minus strains can be identified by screening asporogenous mutants in shake flask media and selecting those mutants that are still intact and contain toxin crystals at the end of the fermentation. Lysis minus strains are suitable for a cell treatment process that will yield a protected, encapsulated toxin protein.

[0075] To prepare a phage resistant variant of said asporogenous mutant, an aliquot of the phage lysate is spread onto nutrient agar and allowed to dry. An aliquot of the phage sensitive bacterial strain is then plated directly over the dried lysate and allowed to dry. The plates are incubated at 30°C. The plates are incubated for 2 days and, at that time, numerous colonies could be seen growing on the agar. Some of these colonies are picked and subcultured onto nutrient agar plates. These apparent resistant cultures are tested for resistance by cross streaking with the phage lysate. A line of the phage lysate is streaked on the plate and allowed to dry. The presumptive resistant cultures are then streaked across the phage line. Resistant bacterial cultures show no lysis anywhere in the streak across the phage line after overnight incubation at 30°C. The resistance to phage is then reconfirmed by plating a lawn of the resistant culture onto a nutrient agar plate. The sensitive strain is also plated in the same manner to serve as the positive control. After drying, a drop of the phage lysate is placed in the center of the plate and allowed to dry. Resistant cultures showed no lysis in the area where the phage lysate has been placed after incubation at 30°C for 24 hours.

[0076] All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety to the extent they are not inconsistent with the explicit teachings of this specification.

[0077] Following are examples which illustrates procedures for practicing the invention. These examples should not be construed as limiting.

Example 1 - Bioassay of Cry1F against black cutworm (*Agrotis ipsilon;* Lepidoptera: Noctuidae) larvae

[0078] Biological activity of Cry1F against black cutworm (BCW) larvae was determined using standard bioassay procedures using MR872 (a *Pseudomonas fluorescens* clone expressing a *cry*1Fa/*cry*1Ab chimeric gene disclosed in U.S. Paatent No. 5,840,554) lyophilized powder preparations. BCW bioassays were conducted by incorporating test samples into artificial diet and challenging larvae with treated and untreated diet. All assays were conducted with BCW artificial diet (BioServ Corporation, Frenchtown, NJ). Diet incorporation tests were conducted by mixing the samples with artificial diet (cooled first to 55°C or below) at a rate of 6 mL suspension plus 54 mL diet. Multiple treatment concentrations were generated by serial dilution. After vortexing, this mixture was poured into plastic trays with compartmentalized 3-ml (Nutrend Container Corporation, Jacksonville, FL) at a rate of approximately 30 ml diet per 24-well tray (each well filled halfway). Nutrend trays with larger wells were used for bioassays with insects in the third instar or older; these also are filled approximately halfway with artificial diet. A water blank containing no test material served as the control. Following at least 30 minutes to allow diet to cool and set, larvae (French Ag Resources, Lamberton,

MN) were placed onto the diet mixture, one larva per well. Wells were then sealed with Mylar sheeting (ClearLam Packaging, IL) using a tacking iron, and several pinholes were made in each well to provide gas exchange. Larvae were held at 25°C for 6 days in a 14:10 (light:dark) holding room. Mortality and stunting were recorded after approximately six days. For LC_{50} bioassays, a minimum of 3 replicates were run, each with 5-7 doses and approximately 24 insects/dose. Results are shown in Table 2 (Bioassays against first- and third-instar *Agrotis ipsilon* larvae with MR872 Cry1F lyophilized powder preparation).

Table 2

Instar	# test dates	LC_{10}	LC_{50}	LC_{90}	slope	# test insects	# control insects	% control mortality
1	5	9	46	249	1.8	790	220	6
3	3	4	17	67	2.2	249	75	0

Example 2 - Efficacy of Cry1F bait against black cutworm larvae

[0079] Cry1F was tested for activity against black cutworm larvae using a bait formulation. MR872 clones were mixed with commercial bait (SoilServ, Salinas, CA) and the resulting mixture was spread across the soil surface of potted wheat seedlings (Feekes growth stage 1.5) at a rate of 50 lb bait/acre. Two concentrations of Cry1F were tested by adding different amounts of toxin to the bait, yielding rates equivalent to 6.25g and 12.5 g of Cry1F toxin/acre. The plants were then infested with 3rd or 4th instar larvae at a rate of approximately 1 insect per 5 plants, and plant damage was measured after 24-48 hours. The damage rating scale runs from 0-4 with 0 being plants cut totally to the ground, and 4 being no visible damage. Controls included plants with no bait added ("no bait"), bait added but without toxin ("blank bait"), and plants with no bait or insects ("control"). Results are shown in Figures 1 and 2.

Example 3 — Efficacy of Transgenic *Cry*1F Sunflower Plants Against *Agrotis ypsilon* as Measured by Insect Growth and Mortality, and Plant Damage

[0080]

Plant treatments:

- Control seedlings at V2 stage
- *B.t.* seedlings at V2 stage (donors with fixed gene pattern); these plants expressed a gene substantially as shown in SEQ ID NO:3.

Insect: *Agrotis ypsilon.* Second instar larvae (third laboratory generation).
Trial: *B.t.* seedlings: five repetitions, five seedlings per repetition, one larva per seedling. Control: five repetitions, five seedlings per repetition, one larva per seedling.
Protocol:

- Each repetition consisted of five seeds that were planted in transparent rectangular trays (15cm x 25cm) and covered to allow confinement of larval insects.
- Each repetition was infested with five larvae. Trays were kept in a chamber at 25 °C and 75% humidity. Evaluation was done at 24, 48, 72, and 96 hours after infestation.
- Evaluation

    1. Plants — categorized as undamaged, damaged (bitten stem, cotyledons or leaves) or cut below the cotyledons. A rating was also given to each repetition: 1 (undamaged plants or with the stem with some bites) to 9 (all plants cut and more than 80% of eaten tissue).
    2. Insects — 96 hours after infestation dead larvae were counted, and surviving larvae were weighed.
    3. Data were analyzed via contingency table analysis.

Results: Pictures are available that show repetition four (48 and 96 hours after infestation). Data are reported in the following tables, which show evaluation of repetitions 24, 48, 72 and 96 hours after infestation with *Agrotis ypsilon.*

Table 3.

24 hours after infestation.								
Rep.	No. Undamaged Plants		No. Damaged Plants		No. Cut Plants		Rating	
	B.t.	control	*B.t.*	control	*B.t.*	control	*B.t.*	control
1	2	2	2	0	1	3	1	5
2	3	3	0	1	2	1	3	4
3	3	2	2	0	0	3	2	5
4	3	1	0	2	0	2	2	3
5	4	2	0	2	1	1	2	3

Table 4.

48 hours after infestation.								
Rep.	No. Undamaged Plants		No. Damaged Plants		No. Cut Plants		Rating	
	B.t.	control	*B.t.*	control	*B.t.*	control	*B.t.*	control
1	2	1	1	1	2	3	3	7
2	2	0	1	2	2	3	3	7
3	1	1	1	0	3	4	3	7
4	2	1	0	0	3	4	4	7
5	2	1	1	1	2	3	3	7

Table 5.

72 hours after infestation.								
Rep.	No. Undamaged Plants		No. Damaged Plants		No. Cut Plants		Rating	
	B.t.	control	*B.t.*	control	*B.t.*	control	*B.t.*	control
1	2	0	1	0	3	5	3	9
2	0	0	3	0	2	5	4	9
3	1	0	1	0	3	5	5	9
4	1	0	0	0	4	5	6	9
5	2	0	0	0	5	5	5	9

Table 6.

96 hours after infestation.									
Rep.	No. Undamaged Plants		No. Damaged Plants		No. Cut Plants		Rating		No. Dead Larvae
	B.t.	control	*B.t.*	control	*B.t.*	control	*B.t.*	control	
1	3	0	0	0	2	5	3	9	4
2	0	0	2	0	3	5	7	9	0

Table 6. (continued)

96 hours after infestation.									
Rep.	No. Undamaged Plants		No. Damaged Plants		No. Cut Plants		Rating		No. Dead Larvae
	B.t.	control	*B.t.*	control	*B.t.*	control	*B.t.*	control	
3	1	0	0	0	4	5	5	9	0
4	0	0	0	0	5	5	6	9	0
5	1	0	0	0	4	5	6	9	0

Table 7.

Larval weight (mg) 96 hours after infestation*							
Repetition		Larvae per Repetition					
		1	2	3	4	5	Mean
2	*B.t.*	100	95	65	115	75	90
	Control	230	170	205	175	175	191
3	*B.t.*	150	135	85	115	120	121
	Control	215	200	90	120	160	157
4	*B.t.*	110	80	110	85	150	107
	Control	170	160	185	200	155	174
5	*B.t.*	120	65	95	60	45	77
	Control	120	190	210	255	110	177

*Four repetitions because in one repetition larvae died

[0081] Conclusion: The *B.t.* seedlings show activity towards *Agrotis ypsilon* at a high level of infestation (1 larva/seedling). This activity is sufficient to adversely affect the growth of the cutworms so that control is achieved.

Example 4 — Black Cutworm (BCW) Control with New Cry1F Maize Events

[0082] Summary. The ability of ten Cry1F maize events (expressing substantially the polynucleotide of SEQ ID NO: 3) to control Black Cutworm (*Agrotis ipsilon* (Hufnagel)) under field conditions was compared to two non-Bt hybrids (Control 1 and Control 2), a hybrid expressing *Cry*1Ab, and a hybrid expressing the *Cry*9C protein for purposes of comparison. All the *Cry*1F events provided good protection against stand loss in comparison to the non-Bt hybrids.

[0083] Materials and methods. Twenty-five seeds for each of the entries were planted using an Almaco cone planter on Day 1. Four replications were planted — each randomized separately. Rows were in pairs, with an unplanted walkway between each pair of rows. Each plot was enclosed in a galvanized steel barrier that is 8 inches high, 2.5 feet wide and 6 feet long. The barriers were pounded into the ground about 2 inches deep on Days 13 and 14. The rims of the barriers were treated with petroleum jelly to discourage larval escape. Wheat straw was scattered inside the barriers to provide shelter for the cutworms. Shortly after emergence, entry plants with *Cry*1F were tested with ELISA strips and the stands were thinned to 10 plants by the removal of non-expressing plants or surplus expressing plants. In three of the plots, the final stand was 8 or 9 plants. The non-*Cry*1F's were also thinned to 10 plants. A small plastic stake was placed behind each plant so that it would be apparent that a plant had disappeared, if this occurred. On day 15, when the plants reached late V1 or early V2, three third instar larval black cutworms were placed by each plant. A second infestation of 2 fourth instar BCW per plant was made on Day 19.

[0084] Plots were examined each day from Day 16 to Day 26. An additional rating was made on Day 29, and the final rating was made on Day 34. Any plant that received damage was marked with a small plastic stake in front of it and the type of damage was recorded on a data sheet. At the end of the trial the final stand count was taken.

[0085] Results. A moderate level of black cutworm pressure was achieved in this trial, resulting in stand reduction in some of the non-Bt entries. For unknown reasons, there was a tendency in this trial for the BCW to dead-heart the

plants instead of cutting them. For this reason, the data on damaged plants were also compared.

[0086] Table 8 shows plant type and the percent Stand Reduction (%SR) and percent damaged plants (%Dam) for each type of plant. Stand Reduction means that the plant was killed. Damaged plants had obvious feeding damage but did not lose their growing point.

Table 8.

Treatment/*Cry*1F event	%SR	%Dam
Non-*B.t.* Control 1	47.5	60
Non-*B.t.* Control 2	32.5	45
*Cry*9C	7.5	20
*Cry*1Ab	15	43
308	2.5	5
188	0	5
218	7.5	15
386	7.5	15
538	2.5	18
778	2.5	15
366	3.1	11
663	5	18
058	2.8	9
227	0	5

[0087] These results are shown graphically in Figures 3 and 4.

[0088] Although no entries were without damage, this is not surprising because the caterpillars have to eat the plants to die. However, in summary, photographs are available that clearly show slightly affected *Cry*1F plants next to completely cut control plants.

Example 5 - Insertion of Toxin Genes Into Plants

[0089] One aspect of the subject invention is the transformation of plants with the subject polynucleotide sequences encoding insecticidal toxins. The transformed plants are resistant to attack by the target pest. Preferred genes for use according to the subject invention are optimized for use in plants. Examples of cutworm-active toxins and genes for use according to the subject invention are shown in SEQ ID NOS. 1-8. The protein and gene of SEQ ID NOS. 1 and 2 are preferred.

[0090] Obviously, a promoter region capable of expressing the gene in a plant is needed. Thus, for *in planta* expression, the DNA of the subject invention is under the control of and operably linked to an appropriate promoter region. Techniques for obtaining *in planta* expression by using such constructs is known in the art.

[0091] Genes encoding pesticidal toxins, as disclosed herein, can be inserted into plant cells using a variety of techniques which are well known in the art. For example, a large number of cloning vectors comprising a replication system in *E. coli* and a marker that permits selection of the transformed cells are available for preparation for the insertion of foreign genes into higher plants. The vectors comprise, for example, pBR322, pUC series, M13mp series, pACYC184, etc. Accordingly, the sequence encoding the *B.t.* toxin can be inserted into the vector at a suitable restriction site. The resulting plasmid is used for transformation into *E. coli.* The *E. coli* cells are cultivated in a suitable nutrient medium, then harvested and lysed. The plasmid is recovered. Sequence analysis, restriction analysis, electrophoresis, and other biochemical-molecular biological methods are generally carried out as methods of analysis. After each manipulation, the DNA sequence used can be cleaved and joined to the next DNA sequence. Each plasmid sequence can be cloned in the same or other plasmids.

[0092] Depending on the method of inserting desired genes into the plant, other DNA sequences may be necessary. If, for example, the Ti or Ri plasmid is used for the transformation of the plant cell, then at least the right border, but often the right and the left border of the Ti or Ri plasmid T-DNA, has to be joined as the flanking region of the genes to be inserted. The use of T-DNA for the transformation of plant cells has been intensively researched and sufficiently

described in EP 120 516; Hoekema (1985) In: *The Binary Plant Vector System*, Offset-durkkerij Kanters B.V., Alblasserdam, Chapter 5; Fraley *et al., Crit. Rev. Plant Sci.* 4:1-46; and An *et al.* (1985) *EMBO J.* 4:277-287.

[0093] Once the inserted DNA has been integrated in the genome, it is relatively stable there and, as a rule, does not come out again. It normally contains a selection marker that confers on the transformed plant cells resistance to a biocide or an antibiotic, such as kanamycin, G 418, bleomycin, hygromycin, or chloramphenicol, *inter alia.* The individually employed marker should accordingly permit the selection of transformed cells rather than cells that do not contain the inserted DNA.

[0094] A large number of techniques are available for inserting DNA into a plant host cell. Those techniques include transformation with T-DNA using *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* as transformation agent, fusion, injection, biolistics (microparticle bombardment), or electroporation as well as other possible methods. If Agrobacteria are used for the transformation, the DNA to be inserted has to be cloned into special plasmids, namely either into an intermediate vector or into a binary vector. The intermediate vectors can be integrated into the Ti or Ri plasmid by homologous recombination owing to sequences that are homologous to sequences in the T-DNA. The Ti or Ri plasmid also comprises the *vir* region necessary for the transfer of the T-DNA. Intermediate vectors cannot replicate themselves in Agrobacteria. The intermediate vector can be transferred into *Agrobacterium tumefaciens* by means of a helper plasmid (conjugation). Binary vectors can replicate themselves both in *E. coli* and in Agrobacteria. They comprise a selection marker gene and a linker or polylinker which are framed by the right and left T-DNA border regions. They can be transformed directly into Agrobacteria (Holsters *et al.* [1978] *Mol. Gen. Genet.* 163:181-187). The *Agrobacterium* used as host cell is to comprise a plasmid carrying a *vir* region. The *vir* region is necessary for the transfer of the T-DNA into the plant cell. Additional T-DNA may be contained. The bacterium so transformed is used for the transformation of plant cells. Plant explants can advantageously be cultivated with *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* for the transfer of the DNA into the plant cell. Whole plants can then be regenerated from the infected plant material (for example, pieces of leaf, segments of stalk, roots, but also protoplasts or suspension-cultivated cells) in a suitable medium, which may contain antibiotics or biocides for selection. The plants so obtained can then be tested for the presence of the inserted DNA. No special demands are made of the plasmids in the case of injection and electroporation. It is possible to use ordinary plasmids, such as, for example, pUC derivatives.

[0095] The transformed cells grow inside the plants in the usual manner. They can form germ cells and transmit the transformed trait(s) to progeny plants. Such plants can be grown in the normal manner and crossed with plants that have the same transformed hereditary factors or other hereditary factors. The resulting hybrid individuals have the corresponding phenotypic properties.

SEQUENCE LISTING

[0096]

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Pro Leu Asp Ile Ser Leu Ser Leu Thr Arg Phe Leu Leu Ser Glu Phe
        35                  40                  45

Val Pro Gly Val Gly Val Ala Phe Gly Leu Phe Asp Leu Ile Trp Gly
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Gly Gln Gly Trp Gly Leu Asp Ile Ala Thr Val Asn Asn His Tyr Asn
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Tyr Asn Gln Gly Leu Glu Asn Leu Arg Gly Thr Asn Thr Arg Gln Trp
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Val Ser Ser Arg Asn Thr Ala Gly Asn Arg Ile Asn Phe Pro Ser Tyr
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Tyr Asn Gln Gly Leu Glu Asn Leu Arg Gly Thr Asn Thr Arg Gln Trp
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Phe Gly Asn Pro His Tyr Val Leu Gly Leu Arg Gly Val Ala Phe Gln
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Gln Thr Gly Thr Asn His Thr Arg Thr Phe Arg Asn Ser Gly Thr Ile
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Thr His Arg Ser Ala Thr Pro Thr Asn Thr Ile Asp Pro Glu Arg Ile
    450                 455                 460

Thr Gln Ile Pro Leu Val Lys Ala His Thr Leu Gln Ser Gly Thr Thr
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Val Val Arg Gly Pro Gly Phe Thr Gly Gly Asp Ile Leu Arg Arg Thr
                485                 490                 495

Ser Gly Gly Pro Phe Ala Tyr Thr Ile Val Asn Ile Asn Gly Gln Leu
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Pro Gln Arg Tyr Arg Ala Arg Ile Arg Tyr Ala Ser Thr Thr Asn Leu
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Claims

1. A method for controlling a cutworm pest wherein said method comprises contacting said pest with a *Cry*1F toxin.

2. The method of claim 1 wherein said toxin is a *Cry*1Fa toxin.

3. The method of claim 1 wherein said toxin is a truncated toxin.

4. The method of claim 1 wherein said toxin is in a plant cell that produced said toxin and wherein said pest contacts said toxin by ingesting said plant cell.

5. The method of claim 1 wherein said cutworm pest is of the genus *Agrotis*.

6. The method of claim 1 wherein said cutworm pest is an *Agrotis ipsilon*.

7. The method of claim 1 wherein said cutworm pest is an *Agrotis malefida*.

8. The method of claim 1 wherein said cutworm pest is of the genus *Porosagrotis*.

9. The method of claim 1 wherein said cutworm pest is a *Porosagrotis gypaetiana*.

10. The method of claim 1 wherein said cutworm pest is of the genus *Xylomyges*.

11. The method of claim 1 wherein said cutworm pest is a *Xylomyges curialis*.

12. The method of claim 1 wherein said cutworm pest is of the Tribe Agrotini

13. The method of claim 1 wherein said cutworm pest is of the genus *Feltia*.

14. The method of claim 1 wherein said cutworm pest is a *Feltia jaculifera*.

15. The method of claim 1 wherein said cutworm pest is of the genus *Euxoa*.

16. The method of claim 1 wherein said cutworm pest is an *Euxoa messoria*.

17. The method of claim 1 wherein said cutworm pest is an *Euxoa scandens*.

18. The method of claim 1 wherein said cutworm pest is an *Euxoa auxiliaris*.

19. The method of claim 1 wherein said cutworm pest is an *Euxoa detersa*.

20. The method of claim 1 wherein said cutworm pest is an *Euxoa tessellata*.

21. The method of claim 1 wherein said cutworm pest is an *Euxoa ochrogaster*.

22. The method of claim 1 wherein said cutworm pest is of the genus *Peridroma*.

23. The method of claim 1 wherein said cutworm pest is *a Peridroma saucia*.

24. The method of claim 4 wherein said plant is a corn plant.

25. The method of claim 4 wherein said plant is a sunflower plant.

26. The method of claim 4 wherein said plant is a soybean plant.

27. The method of claim 4 wherein said plant is a canola plant.

28. The method of claim 4 wherein said plant is a cotton plant.

29. The method of claim 4 wherein said contacting step is preceded by the step of planting a seed for a plant that comprises a polynucleotide that encodes said toxin.

Patentansprüche

1. Verfahren zur Bekämpfung eines Eulenfalterraupen ("Cutworm")-Schädlings, wobei das Verfahren das Kontaktieren des Schädlings mit einem *Cry*1F-Toxin umfasst.

2. Verfahren nach Anspruch 1, worin das Toxin ein *Cry*1Fa-Toxin ist.

3. Verfahren nach Anspruch 1, worin das Toxin ein verkürztes Toxin ist.

4. Verfahren nach Anspruch 1, worin das Toxin in einer Pflanzenzelle vorliegt, welche das Toxin produzierte, und worin der Schädling durch Aufnahme der Pflanzenzelle in Kontakt mit dem Toxin kommt.

5. Verfahren nach Anspruch 1, worin der Eulenfalterraupen-Schädling aus der Gattung *Agrotis* ist.

6. Verfahren nach Anspruch 1, worin der Eulenfalterraupen-Schädling ein *Agrotis ipsilon* ist.

7. Verfahren nach Anspruch 1, worin der Eulenfalterraupen-Schädling ein *Agrotis malefida* ist.

8. Verfahren nach Anspruch 1, worin der Eulenfalterraupen-Schädling aus der Gattung *Porosagrotis* ist.

9. Verfahren nach Anspruch 1, worin der Eulenfalterraupen-Schädling ein *Porosagrotis gypaetiana* ist.

10. Verfahren nach Anspruch 1, worin der Eulenfalterraupen-Schädling aus der Gattung *Xylomyges* ist.

11. Verfahren nach Anspruch 1, worin der Eulenfalterraupen-Schädling ein *Xylomyges curialis* ist.

12. Verfahren nach Anspruch 1, worin der Eulenfalterraupen-Schädling aus dem Stamm Agrotini ist.

13. Verfahren nach Anspruch 1, worin der Eulenfalterraupen-Schädling aus der Gattung *Feltia* ist.

14. Verfahren nach Anspruch 1, worin der Eulenfalterraupen-Schädling ein *Feltia jaculifera* ist.

15. Verfahren nach Anspruch 1, worin der Eulenfalterraupen-Schädling aus der Gattung *Euxoa* ist.

16. Verfahren nach Anspruch 1, worin der Eulenfalterraupen-Schädling ein *Euxoa messoria* ist.

17. Verfahren nach Anspruch 1, worin der Eulenfaiterraupen-Schädling ein *Euxoa scandens* ist.

18. Verfahren nach Anspruch 1, worin der Eulenfalterraupen-Schädling ein *Euxoa auxiliaris* ist.

19. Verfahren nach Anspruch 1, worin der Eulenfalterraupen-Schädling ein *Euxoa detersa* ist.

20. Verfahren nach Anspruch 1, worin der Eulenfalterraupen-Schädling ein *Euxoa tessellata* ist.

21. Verfahren nach Anspruch 1, worin der Eulenfalterraupen-Schädling ein *Euxoa ochrogaster* ist.

22. Verfahren nach Anspruch 1, worin der Eulenfalterraupen-Schädling aus der Gattung *Peridroma* ist.

23. Verfahren nach Anspruch 1, worin der Eulenfalterraupen-Schädling ein *Peridroma saucia* ist.

24. Verfahren nach Anspruch 4, worin die Pflanze eine Getreidepflanze ist.

25. Verfahren nach Anspruch 4, worin die Pflanze eine Sonnenblumenpflanze ist.

26. Verfahren nach Anspruch 4, worin die Pflanze eine Sojabohnenpflanze ist.

27. Verfahren nach Anspruch 4, worin die Pflanze eine Canolapflanze ist.

28. Verfahren nach Anspruch 4, worin die Pflanze eine Baumwollpflanze ist.

29. Verfahren nach Anspruch 4, worin dem Kontaktierungsschritt der Schritt des Pflanzens eines Samens für eine Pflanze vorausgeht, die ein Polynukleotid umfasst, welches für das Toxin kodiert.

Revendications

1. Procédé de lutte contre un nuisible ver gris où ledit procédé comprend la mise en contact dudit nuisible avec une toxine *Cry*1F.

2. Procédé selon la revendication 1 où ladite toxine est une toxine *Cry*1Fa.

3. Procédé selon la revendication 1 où ladite toxine est une toxine tronquée.

4. Procédé selon la revendication 1 où ladite toxine est dans une cellule végétale qui a produit ladite toxine et où ledit nuisible vient en contact avec ladite toxine en ingérant ladite cellule végétale.

5. Procédé selon la revendication 1 où ledit nuisible ver gris est du genre *Agrotis*.

6. Procédé selon la revendication 1 où ledit nuisible ver gris est un *Agrotis ipsilon*.

7. Procédé selon la revendication 1 où ledit nuisible ver gris est un *Agrotis malefida*.

8. Procédé selon la revendication 1 où ledit nuisible ver gris est du genre *Porosagrotis*.

9. Procédé selon la revendication 1 où ledit nuisible ver gris est un *Porosagrotis gypaetiana*.

10. Procédé selon la revendication 1 où ledit nuisible ver gris est du genre *Xylomyges*.

11. Procédé selon la revendication 1 où ledit nuisible ver gris est un *Xylomyges curialis*.

12. Procédé selon la revendication 1 où ledit nuisible ver gris est de la tribu Agrotini.

13. Procédé selon la revendication 1 où ledit nuisible ver gris est du genre *Feltia*.

14. Procédé selon la revendication 1 où ledit nuisible ver gris est un *Feltia jaculifera*.

15. Procédé selon la revendication 1 où ledit nuisible ver gris est du genre *Euxoa*.

16. Procédé selon la revendication 1 où ledit nuisible ver gris est un *Euxoa messoria*.

17. Procédé selon la revendication 1 où ledit nuisible ver gris est un *Euxoa scandens*.

18. Procédé selon la revendication 1 où ledit nuisible ver gris est un *Euxoa auxiliaris*.

19. Procédé selon la revendication 1 où ledit nuisible ver gris est un *Euxoa detersa*.

20. Procédé selon la revendication 1 où ledit nuisible ver gris *est* un *Euxoa tessellata*.

21. Procédé selon la revendication 1 où ledit nuisible ver gris est un *Euxoa ochrogaster*.

22. Procédé selon la revendication 1 où ledit nuisible ver gris est du genre *Peridroma*.

23. Procédé selon la revendication 1 où ledit nuisible ver gris est un *Peridroma saucia*.

24. Procédé selon la revendication 4 où ladite plante est une plante de type maïs.

25. Procédé selon la revendication 4 où ladite plante est une plante de type tournesol.

26. Procédé selon la revendication 4 où ladite plante est une plante de type soja.

27. Procédé selon la revendication 4 où ladite plante est une plante de type colza.

28. Procédé selon la revendication 4 où ladite plante est une plante de type coton.

29. Procédé selon la revendication 4 où ladite étape de mise en contact est précédée par l'étape de plantation d'une graine pour une plante qui comprend un polynucléotide qui code ladite toxine.

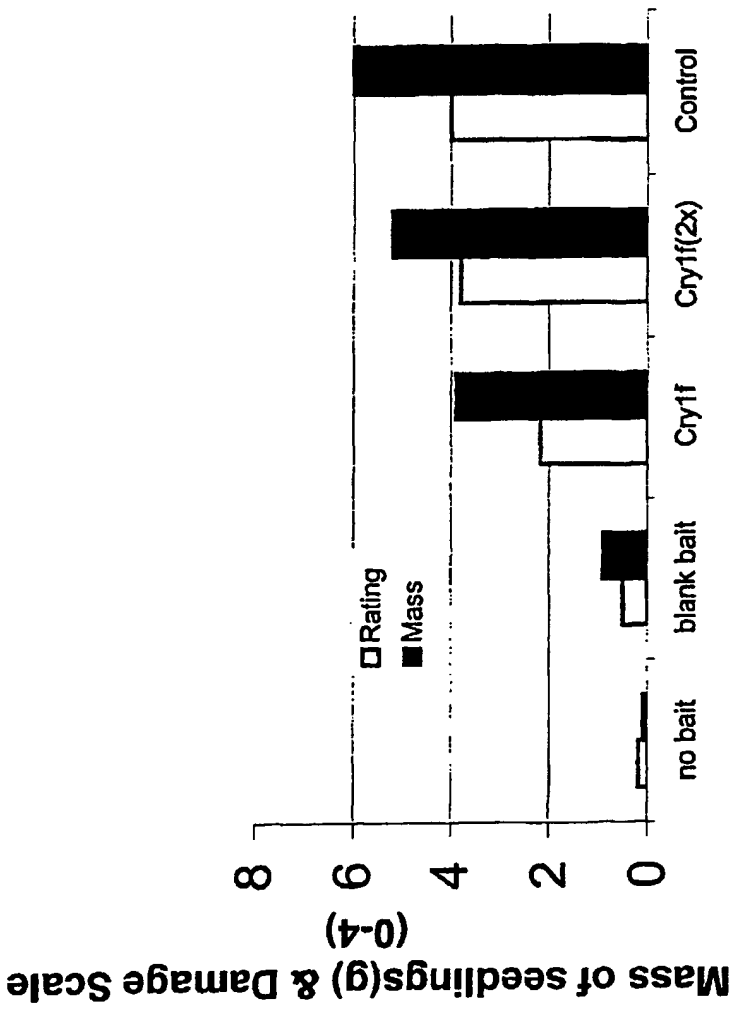
Mass of seedlings(g) & Damage Scale
(0-4)
8
6
4
2
0
Rating
Mass
no bait
blank bait
Cry1f
Cry1f(2x)
Control

FIG. 1

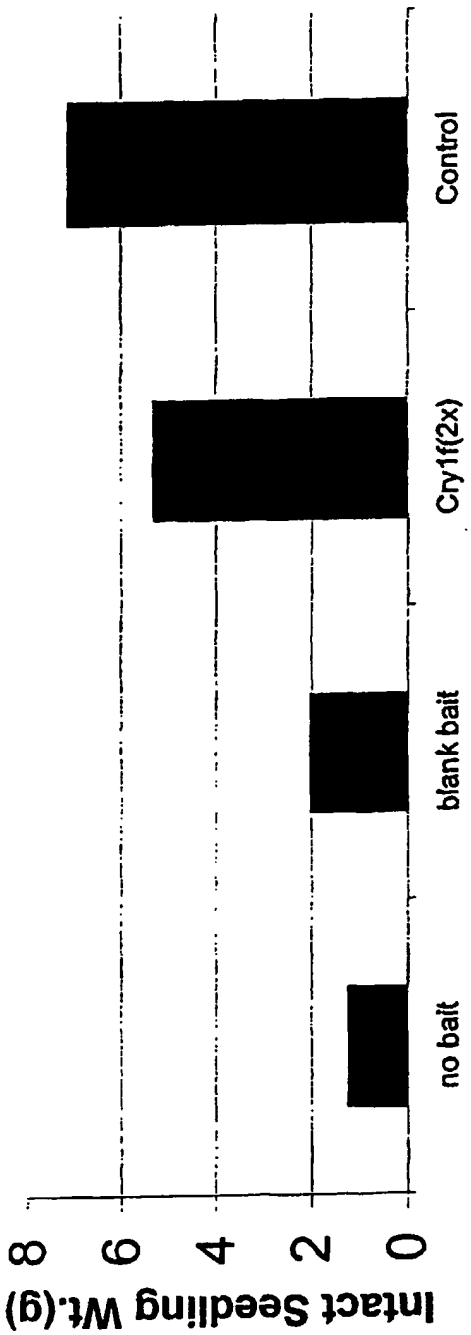
Intact Seedling Wt.(g)
0
2
4
6
8
no bait
blank bait
Cry1f(2x)
Control

FIG. 2

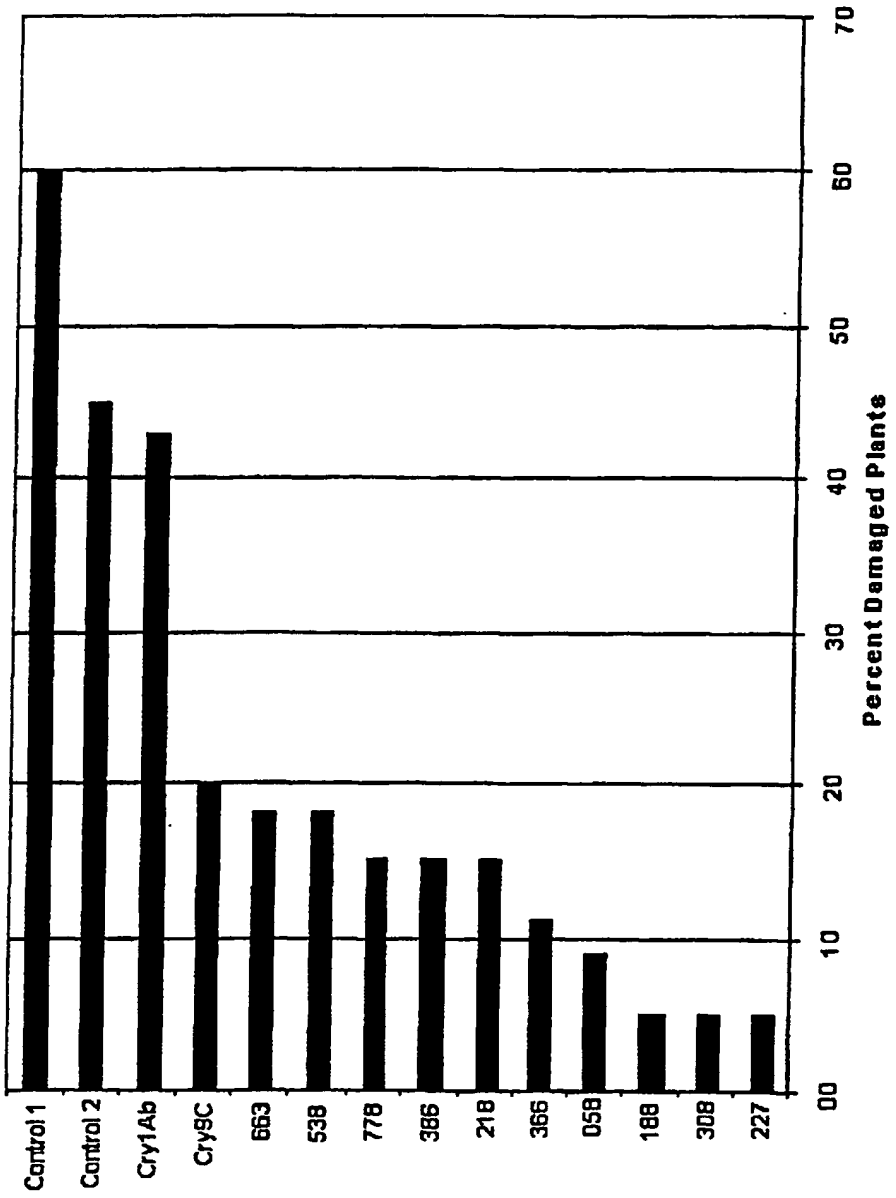
Control 1
Control 2
Cry1Ab
Cry9C
663
538
778
386
218
366
058
188
308
227
00
10
20
30
40
50
60
70
Percent Damaged Plants

FIG. 3

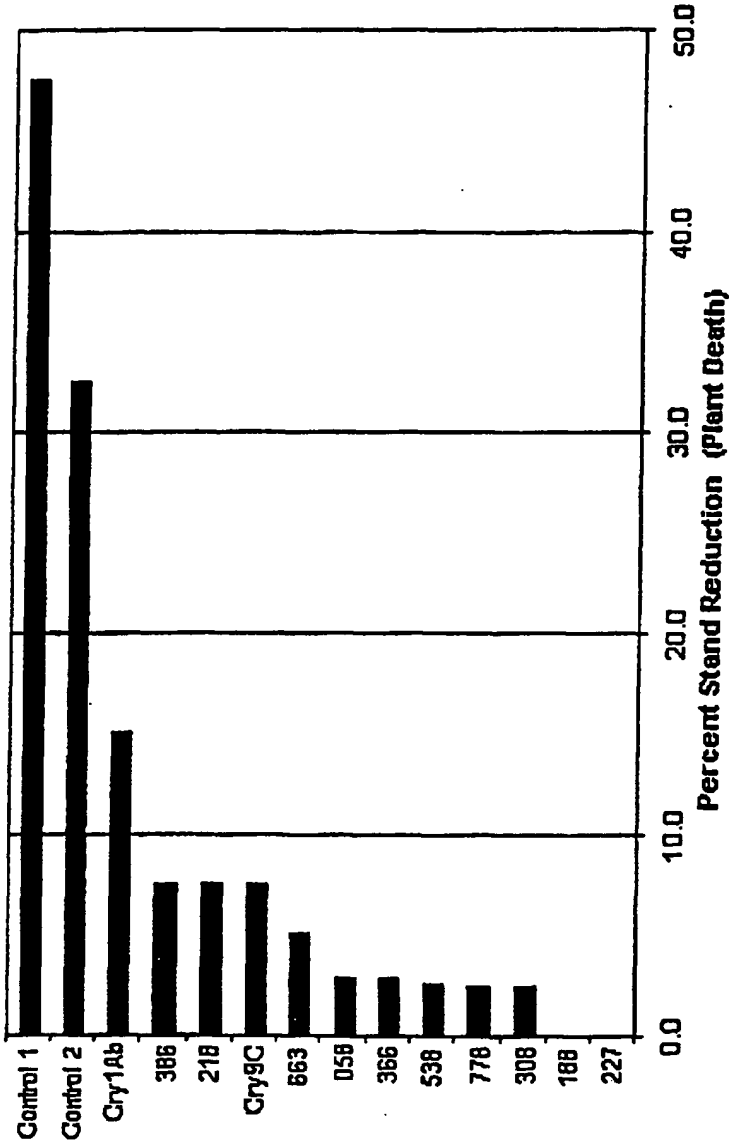
Control 1
Control 2
Cry1Ab
386
218
Cry9C
663
058
366
538
778
308
168
227
0.0
10.0
20.0
30.0
40.0
50.0
Percent Stand Reduction (Plant Death)

FIG. 4